Attorney's Docket No.: 06497-013002

Applicant: James C. Liao Serial No.: 10/048,186 Filed: June 19, 2002

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Amendments to the Claims:

All amendments and cancellations to the claims are made without prejudice or disclaimer. This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

- 1. (currently amended) A bacterial host cell that produces a metabolite, the host cell comprising a nucleic acid sequence comprising a promoter and nucleic acid sequence encoding a biosynthetic enzyme for production of an isoprenoid, a polyketide, or a polyhydroxyalkanoate; the nucleic acid sequence being operably linked to the promoter which is bound by ntrC; the host cell being genetically modified by deletion or inactivating mutation in glnL, wherein the bacterial host cell is an *E. coli* cell.
 - 2.-4. (cancelled)
 - 5. (previously presented) The host cell of claim 1 wherein the promoter is glnAp2.
 - 6.-10. (cancelled)
- 11. (withdrawn currently amended) The host cell of claim [[10]] 17 wherein the isoprenoid is a carotenoid.
- 12. (currently amended) The host cell of claim [[10]] $\underline{17}$ wherein the isoprenoid is lycopene, β -carotene, astaxanthin, or one of their precursors.

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13. (currently amended) The host cell of claim [[10]] 17 wherein the first enzyme is isopentenyl diphosphate isomerase, geranylgeranyl diphosphate synthase, or 1-deoxyxylulose 5-phosphate synthase.

14.-16. (cancelled)

17. (currently amended) The host cell of claim 10 An E. coli host cell comprising a first expression cassette comprising a promoter and a nucleic acid sequence encoding an enzyme that catalyzes biosynthesis of an isoprenoid; the nucleic acid sequence being operably linked to the glnAp2 promoter which is regulated by acetyl phosphate in the absence of nitrogen starvation, wherein the cell is lacking a functional glaL histidine protein kinase gene.

18.-20. (cancelled)

- 21. (currently amended) The host cell of claim [[10]] <u>17</u> wherein the host cell further contains a nucleic acid sequence encoding a phosphoenolpyruvate synthase.
- 22. (withdrawn currently amended) A method of producing a isoprenoid in a host cell, the method comprising:

providing the host cell of claim [[10]] 17, wherein the first enzyme is a biosynthetic enzyme that catalyzes synthesis of the isoprenoid;

overexpressing a phosphoenolpyruvate synthase; and

expressing the biosynthetic enzymes required for that catalyzes synthesis of the isoprenoid.

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23. (withdrawn – currently amended) A method of producing a lycopene in a bacterial host cell, the method comprising:

providing the host cell of claim [[10]] 17; and

expressing a 1-deoxy-D-xylulose 5-phosphate synthase, a geranylgeranyl diphosphate synthase, a phytoene synthase, and a phytoene desaturase, at least one of which is the first enzyme expressed from the first expression cassette.

24. (currently amended) A kit comprising (i) a nucleic acid sequence containing a promoter bound by ntrC such that the promoter is regulated by acetyl phosphate in a defined bacterial host cell, and a coding sequence that encodes an enzyme for isoprenoid biosynthesis; and (ii) the defined host cell which is an *E. coli* host cell genetically modified by deletion or inactivating mutation of the glnL gene.

25.- 36. (cancelled)

- 37. (withdrawn) The host cell of claim 1 wherein the heterologous metabolite is a polyketide.
- 38. (withdrawn) The host cell of claim 1 wherein the heterologous metabolite is a polyhydroxyalkanoate.
 - 39. (cancelled)
 - 40. (currently amended) A E. coli E. coli bacterial host cell comprising:
 - (i) a genetic alteration inactivating the glnL gene; and
- (ii) a nucleic acid sequence comprising a coding sequence encoding a biosynthetic enzyme that catalyzes production of an isoprenoid, polyketide, or polyhdroxyalkanoate, and an operably linked promoter that is bound by ntrC and <u>regulated by</u> acetyl phosphate.

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41. (previously presented) The host cell of claim 40 wherein the biosynthetic enzyme is isopentenyl diphosphate isomerase, geranylgeranyl diphosphate synthase, 1-deoxyxylulose 5-phosphate synthase, or phosphoenolpyruvate synthase.

- 42. (cancelled)
- 43. (cancelled)
- 44. (cancelled)
- 45. (currently amended) The kit of claim [[42]] <u>24</u> wherein the promoter is the <u>glnAp2</u> promoter.
- 46. (withdrawn) A method of producing a metabolite in a bacterial host cell, the method comprising:

providing the host cell of claim 1; and culturing the host cell under conditions such that acetyl phosphate triggers the promoter.

- 47. (withdrawn) The method of claim 46 in which the culturing comprises nitrogen rich conditions.
- 48. (withdrawn) The method of claim 46 in which the culturing comprises growth to late logarithmic growth.
- 49. (withdrawn) The method of claim 46 in which the culturing comprises growth to stationary phase.

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50. (withdrawn) The method of claim 48 in which the metabolite is lycopene, the promoter is glnAp2, and at least 5 mg L⁻¹ of lycopene are produced.

- 51. (cancelled)
- 52. (withdrawn currently amended) The host cell of claim [[10]] 12 wherein the isoprenoid is lycopene.
 - 53. (new) The host cell of claim 12 wherein the isoprenoid is β -carotene.
 - 54. (new) The host cell of claim 12 wherein the isoprenoid is astaxanthin.
- 55. (new) The host cell of claim 13 wherein the enzyme is isopentenyl diphosphate isomerase.
- 56. (new) The host cell of claim 13 wherein the enzyme is geranylgeranyl diphosphate synthase.
- 57. (new) The host cell of claim 13 wherein the enzyme is 1-deoxyxylulose 5-phosphate synthase.
 - 58. (new) The method of claim 46 wherein the metabolite is a polyketide.
 - 59. (new) The method of claim 46 wherein the metabolite is a polyhydroxyalkanoate.
 - 60. (new) The method of claim 46 in which the promoter is glnAp2.

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61. (new) The method of claim 47 in which the promoter is glnAp2.

- 62. (new) The method of claim 48 in which the promoter is glnAp2.
- 63. (new) The method of claim 49 in which the promoter is glnAp2.
- 64. (new) The method of claim 49 in which the metabolite is a carotenoid.
- 65. (new) The method of claim 49 in which the metabolite is lycopene.
- 66. (new) The method of claim 65 in which the culturing comprises nitrogen rich conditions.
- 67. (new) The method of claim 65 in which the culturing comprises growth to late logarithmic growth.
- 68. (new) The method of claim 65 in which the culturing comprises growth to stationary phase.
- 69. (new) The method of claim 58 in which the culturing comprises nitrogen rich conditions.
- 70. (new) The method of claim 58 in which the culturing comprises growth to late logarithmic growth.
- 71. (new) The method of claim 58 in which the culturing comprises growth to stationary phase.

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72. (new) The method of claim 59 in which the culturing comprises nitrogen rich conditions.

- 73. (new) The method of claim 59 in which the culturing comprises growth to late logarithmic growth.
- 74. (new) The method of claim 59 in which the culturing comprises growth to stationary phase.